

## ABSTRACT

Compositions, reaction mixtures, and kits, as well as methods for their use, comprising a pair of FRET hybridization probes hybridizing adjacently to a target nucleic acid sequence, each hybridization probe comprising (i) a nucleotide sequence entity which is substantially complementary to the sequence of the target nucleic acid, (ii) a fluorescent entity, being either a FRET donor entity or a FRET acceptor entity, and (iii) a spacer entity connecting the nucleotide sequence entity and the fluorescent entity. The intensity of fluorescence emission from the FRET donor entity and from the FRET acceptor entity is not substantially affected by quenching activity of nucleotide residues present in the sequence of the target nucleic acid or in the nucleotide sequence entities of the hybridization probes.